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PURIFICATION OF DIHYDROPTEROATE SYNTHETASE FROM MENINGOCOCCAL AND GONOCOCCAL STRAINS AND DEVELOPMENT OF NEW CHEMOTHERAPEUTIC SULFUR DRUGS

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for folic acid biosynthesis, and the synthesis and testing in a cell-free systems as well as in vitro of new and complex sulfur containing drugs.

We have successfully purified one crucial enzyme several fold from different strains of meningococci and gonococci, have developed techniques for the cultivation of these bacteria, and have examined the efficacy of a substantial number of sulfur derivatives, new and old, in cell-free and in vitro systems.

We fell we can safely conclude that the modification of sulfur containing molecules as inhibitors holds exciting chemotherapeutic promise.

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PURIFICATION OF DIHYDROPTEROATE SYNTHETASE FROM MENINGOCOCCAL AND GONOCOCCAL STRAINS AND DEVELOPMENT OF NEW CHEMOTHERAPEUTIC SULFUR DRUGS

SUMMARY

The purpose of this work is to provide a rational chemotherapeutic approach to diseases of bacterial origin. It focusses on gonorrhea and meningitis but has wider applicability. The methodology involves molecular considerations and therefore requires the purification of the particular enzymes responsible for folic acid biosynthesis, and the synthesis and testing in a cell-free systems as well as in vitro of new and complex sulfur containing drugs.

We have successfully purified on crucial enzyme several fold from different strains of meningococci and gonococci, have developed techniques for the cultivation of these bacteria, and have examined the efficacy of the substantial number of sulfur derivatives, new and old, in cell-free and in vitro systems.

We fell we can safely conclude that the modification of sulfur containing molecules as inhibitors holds exciting chemotherapeutic promise.

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This past year we have made significant progress with our task of purifying dihydropteroate synthetase from three meningococcal strains of varying sulfa sensitivity. The progress is presented below in the following tables.

Purification of dihydropteroate synthetase from M-166 (MIC=2.0 ug/ml)

Procedure	Volumn ml	Activity units/ml	Total Act. units	Protein mg/ml	Specific Act. units/mg	Yield %	Purificati
Crude	38	33	1254	15	2.2	100	1
0-65% A.S.	35	34.2	1197	9	3.8	95.46	1.72
Sephadex G-100	65	17.69	1150	0.5	35.4	91.71	16.1
DEAE-Cellulose	110	7.03	773.3	0.05	140.6	61.67	63.9

Purification of dihydropteroate synthetase from M-60 (MIC=0.8 ug/ml)

Crude	20	31.2	624	13	2.4	100	1
0-65% A.S.	10	53	530	10	5.3	85	2.2
Sephadex G-25	8	55.5	444.4	5.5	10.1	71.16	4.2
Sephadex G-100	30	7.02	210.6	0.1	70.2	33.8	29.25

Purification of dihydropteroate synthetase from M-126 (MIC=12.5-25 ug/

Crude	20	28.8	576	18	1.6	100	1
0-65% A.S.	12	34.8	417.6	12	2.9	72.5	1.81
Sephadex G-100	22	18.2	400.4	1	18.2	69.52	11.37

Electrophoresis reveals one protein band and seems promising as a purification step.

We have attempted to purify the synthetase from gonococcal strains as well, but are constrained by the low specific activity of the enzyme in this pathogen.

Purification of dihydropteroate synthetase from Kohn (MIC=8.0 ug/ml)

Procedure	Volumn ml	Activity units/ml	Total Act. units	Protein mg/ml	Specific Act. units/mg	Yield %	Purificatio
Crude	15	37.8	567	18	2.1	100	1
0-65% A.S.	10	45	450	10	4.5	79.37	2.14
DEAE-Oellulose	40	4.5	180	1	30	31.74	14.28

To facilitate the purification, we have been actively engaged in establishing the proper condition for the growth of large quantities of gonococci.

We have determined MIC values for four different gonococcal strains with respect to sulfadiazine.

MIC	mcg/ml
7134	25
Kohn	12.5
A-12	6.25
CDC-9	3.1

A number of chromatographic experiments with Sephadex of varying pore size indicates the synthetase in gonococcal extracts are rather large in molecular dimensions (far larger than meningococcal synthetases).

We have also proceeded with the purification of dihydrofolate reductase from gonococcal extracts. The progress is presented in the following chart.

Purification of N. gonorrhoeae dihydrofolate reductase

Fraction	Total units	Specific Activity
1. Crude extract	321	1.0
2. 1% Streptomycin	280	1.32
3. 55-95% $(NH_4)_2SO_4$	220	5.00
4. Tube No. 22-24 (Sephadex G-100 eluates)	92	64
5. Tube No. 24-26	65	56

This past year, using our accurate radioassay we have investigated the effects of a series of sulfone analogs.

Table Inhibition of partially purified dihydropteroate synthetase by various sulfones and sulfonamides. [a]

Compound	K_I	$(I/S)_{0.5}$ [b]	Reciprocal relative effectiveness (x 100)
p-diaminodiphenylsulfone	7.0×10^{-7} M	0.35	100.0
sulfadiazine	2.0×10^{-6} M	1.0	35.1
4-amino-4'-acetamido-diphenylsulfone	3.3×10^{-6} M	1.65	21.2
sulfanilamide	6.0×10^{-6} M	3.0	11.7
4-amino-4'-formamido-diphenylsulfone	7.5×10^{-6} M	3.75	9.3
4-(p-bromophenylsulfonyl)-aniline	3.6×10^{-5} M	18.3	1.9
p-(4-nitrophenylsulfonyl) aniline	5.5×10^{-5} M	27.5	1.3
4,4'-diacetamidodiphenyl-sulfone	1.1×10^{-4} M	55.0	0.64
4,4'-dihydroxydiphenyl-sulfone	1.6×10^{-4} M	80.0	0.44
m-aminodiphenylsulfone	1.7×10^{-4} M	83.3	0.42
diphenylsulfone	2.2×10^{-4} M	110.0	0.32
4(methylsulfonyl)-phenylhydrazine	2.2×10^{-4} M	110.0	0.32
4,4'-diformamido-diphenylsulfone	2.2×10^{-4} M	110.0	0.32
methylsulfone	no activity	-	-
4-fluorodiphenylsulfone	no activity	-	-
p-fluorophenyl-p-tolyl-sulfone	no activity	-	-
p-fluorophenylphenyl-sulfone	no activity	-	-

a. K_m for dihydropteroate synthetase from N. meningitidis M-166 = 2.0×10^{-6} M.

b. $(I/S)_{0.5}$ is the ratio of inhibitor to substrate giving 50 percent inhibition $[K_I/K_m = (I/S)_{0.5}]$